

WHAT IS CLAIMED IS:

- 1                   1.       A nucleic acid-lipid particle composition for introducing a nucleic acid  
2 into a cell, said particle comprising: a cationic lipid, a conjugated lipid that inhibits  
3 aggregation of particles, a nucleic acid and an endosomal membrane destabilizer.
- 1                   2.       The nucleic acid-lipid particle composition of claim 1, wherein said  
2 endosomal membrane destabilizer is outside said nucleic acid-lipid particle.
- 1                   3.       The nucleic acid-lipid particle composition of claim 1, wherein said  
2 endosomal membrane destabilizer is both outside and inside said nucleic acid-lipid particle.
- 1                   4.       The nucleic acid-lipid particle composition of claim 1, wherein said  
2 endosomal membrane destabilizer is  $\text{Ca}^{++}$  ion.
- 1                   5.       The nucleic acid-lipid particle composition of claim 4, wherein the  
2 concentration of  $\text{Ca}^{++}$  ion is from about 0.1 mM to about 100 mM.
- 1                   6.       The nucleic acid-lipid particle composition of claim 5, wherein the  
2 concentration of  $\text{Ca}^{++}$  ion is from about 1 mM to about 20 mM.
- 1                   7.       The nucleic acid-lipid particle composition of claim 1, wherein said  
2 particle has a median diameter of less than about 150 nm.
- 1                   8.       The nucleic acid-lipid particle composition of claim 1, wherein said  
2 cationic lipid is a member selected from the group consisting of N,N-dioleoyl-N,N-  
3 dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide  
4 (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-  
5 (1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), and N,N-  
6 dimethyl-2,3-dioleoyloxy)propylamine (DODMA), and combinations thereof.
- 1                   9.       The nucleic acid-lipid particle composition of claim 1, wherein said  
2 particle further comprises an additional noncationic lipid.
- 1                   10.      The nucleic acid-lipid particle composition of claim 9, wherein said  
2 noncationic lipid is selected from the group consisting of DOPE, POPC, and EPC.

1                   11.     The nucleic acid-lipid particle composition of claim 1, wherein said  
2 particle comprises a functional group that facilitates  $\text{Ca}^{++}$  ion chelation.

1                   12.     The nucleic acid-lipid particle composition of claim 1, wherein said  
2 conjugated lipid that inhibits aggregation of particles has the formula



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3  
4 wherein:     A is a lipid moiety;  
5               W is a hydrophilic polymer; and  
6               Y is a polycationic moiety.

1                   13.     The nucleic acid-lipid particle composition of claim 12, wherein W is a  
2 polymer selected from the group consisting of PEG, polyamide, polylactic acid, polyglycolic  
3 acid, polylactic acid/polyglycolic acid copolymers and combinations thereof, said polymer  
4 having a molecular weight of about 250 to about 7000 daltons.

1                   14.     The nucleic acid-lipid particle composition of claim 12, wherein Y has  
2 at least 4 positive charges at a selected pH.

1                   15.     The nucleic acid-lipid particle composition of claim 12, wherein Y is a  
2 member selected from the group consisting of lysine, arginine, asparagine, glutamine,  
3 derivatives thereof and combinations thereof.

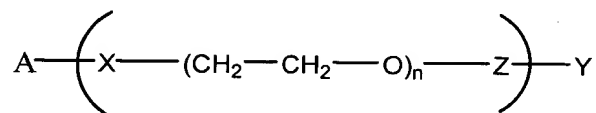
1                   16.     The nucleic acid-lipid particle composition of claim 12, wherein A is a  
2 member selected from the group consisting of a diacylglycerol moiety, a dialkylglycerol  
3 moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane moiety and a 1,2-  
4 dialkyl-3-aminopropane moiety.

1                   17.     The nucleic acid-lipid particle composition of claim 12, wherein W is  
2 PEG.

1                   18.     The nucleic acid-lipid particle composition of claim 12, wherein W is a  
2 polyamide polymer.

1                   19.     The nucleic acid-lipid particle composition of claim 12, wherein W has  
2 a molecular weight of about 250 to about 2000 daltons.

**20.** The nucleic acid-lipid particle composition of claim 17, having the general structure of Formula II:



## II

wherein

X is a member selected from the group consisting of a single bond or a functional group covalently attaching said lipid to at least one ethylene oxide unit;

Z is a member selected from the group consisting of a single bond or a functional group covalently attaching said at least one ethylene oxide unit to a cationic group; and

$n$  is an integer having a value of between about 6 to about 50.

**21.** The nucleic acid-lipid particle composition of claim **20**, wherein X is a member selected from the group consisting of a single bond, phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho, phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

**22.** The nucleic acid-lipid particle composition of claim **20**, wherein Z is a member selected from the group consisting of a single bond, phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho, phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

23. The nucleic acid-lipid particle composition of claim 20, wherein  
A is a diacylglycerol moiety;  
X is phosphoethanolamido;  
Z is NR, wherein R is a hydrogen atom; and  
Y is a member selected from the group consisting of about 1 to about 10 basic amino acids or derivatives thereof.

**24.** The nucleic acid-lipid particle composition of claim 23, wherein

2 A is a diacylglycerolyl moiety having 2 fatty acyl chains, wherein each acyl  
3 chain is independently between 2 and 30 carbons in length and is either saturated or has  
4 varying degrees of saturation.

1 25. The nucleic acid-lipid particle composition of claim 23, wherein  
2 Y is a member selected from the group consisting of lysine, arginine,  
3 asparagine, glutamine, derivatives thereof and combinations thereof.

1 26. The nucleic acid-lipid particle composition of claim 23, wherein  
2 A is a diacylglycerolyl moiety having 2 fatty acyl chains, wherein each acyl  
3 chain is a saturated C-18 carbon chain; and  
4 Y is a cationic group having 4 lysine residues or derivatives thereof.

1 27. The nucleic acid-lipid particle composition of claim 1, wherein said  
2 conjugated lipid that inhibits aggregation of particles is a PEG-lipid.

1 28. The nucleic acid-lipid particle composition of claim 27, wherein said  
2 PEG-lipid is PEG-ceramide.

1 29. The nucleic acid-lipid particle composition of claim 28, wherein the  
2 ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to about 20  
3 carbon atoms.

1 30. The nucleic acid-lipid particle composition of claim 28, wherein said  
2 PEG-lipid is PEG-phosphatidylethanolamine.

1 31. The nucleic acid-lipid particle composition of claim 1, wherein said  
2 conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.

1 32. The nucleic acid-lipid particle composition of claim 1, wherein said  
2 nucleic acid is selected from the group consisting of a plasmid, an antisense oligonucleotide,  
3 and a ribozyme.

1 33. A method of introducing a nucleic acid into a cell, said method  
2 comprising:  
3 contacting said cell with a nucleic acid-lipid particle composition, said particle  
4 comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a  
5 nucleic acid; and an endosomal membrane destabilizer.

1                   **34.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said endosomal membrane destabilizer is outside said nucleic acid-lipid particle.

1                   **35.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said endosomal membrane destabilizer is  $\text{Ca}^{++}$  ion.

1                   **36.**     The method of introducing a nucleic acid into a cell of claim **35**,  
2     wherein the concentration of  $\text{Ca}^{++}$  ion is from about 0.1 mM to about 100 mM.

1                   **37.**     The method of introducing a nucleic acid into a cell of claim **36**,  
2     wherein the concentration of  $\text{Ca}^{++}$  ion is from about 1 mM to about 20 mM.

1                   **38.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said particle has a median diameter of less than about 150 nm.

1                   **39.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said cationic lipid is a member selected from the group consisting of N,N-dioleyl-  
3     N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium  
4     bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride  
5     (DOTAP), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA),  
6     and N,N-dimethyl-2,3-dioleoyloxy)propylamine (DODMA), and combinations thereof.

1                   **40.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said particle further comprises an additional noncationic lipid.

1                   **41.**     The method of introducing a nucleic acid into a cell of claim **40**,  
2     wherein said noncationic lipid is selected from the group consisting of DOPE, POPC, and  
3     EPC.

1                   **42.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said particle comprises a functional group that facilitates  $\text{Ca}^{++}$  ion chelation.

1                   **43.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said conjugated lipid that inhibits aggregation of particles has the formula



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4 wherein: A is a lipid moiety;  
 5 W is a hydrophilic polymer; and  
 6 Y is a polycationic moiety.

1 44. The method of introducing a nucleic acid into a cell of claim 43,  
 2 wherein W is a polymer selected from the group consisting of PEG, polyamide, polylactic  
 3 acid, polyglycolic acid, polylactic acid/polyglycolic acid copolymers and combinations  
 4 thereof, said polymer having a molecular weight of about 250 to about 7000 daltons.

1 45. The method of introducing a nucleic acid into a cell of claim 43,  
 2 wherein Y has at least 4 positive charges at a selected pH.

1 46. The method of introducing a nucleic acid into a cell of claim 43,  
 2 wherein Y is a member selected from the group consisting of lysine, arginine, asparagine,  
 3 glutamine, derivatives thereof and combinations thereof.

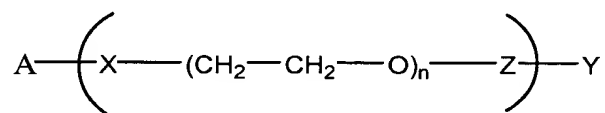
1 47. The method of introducing a nucleic acid into a cell of claim 43,  
 2 wherein A is a member selected from the group consisting of a diacylglycerol moiety, a  
 3 dialkylglycerol moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane  
 4 moiety and a 1,2-dialkyl-3-aminopropane moiety.

1 48. The method of introducing a nucleic acid into a cell of claim 43,  
 2 wherein W is PEG.

1 49. The method of introducing a nucleic acid into a cell of claim 43,  
 2 wherein W is a polyamide polymer.

1 50. The method of introducing a nucleic acid into a cell of claim 43,  
 2 wherein W has a molecular weight of about 250 to about 2000 daltons.

1 51. The method of introducing a nucleic acid into a cell of claim 48,  
 2 having the general structure of Formula II:



II

4 wherein

X is a member selected from the group consisting of a single bond or a functional group covalently attaching said lipid to at least one ethylene oxide unit;  
Z is a member selected from the group consisting of a single bond or a functional group covalently attaching said at least one ethylene oxide unit to a cationic group;  
and  
n is an integer having a value of between about 6 to about 50.

**52.** The method of introducing a nucleic acid into a cell of claim **51**,  
wherein

X is a member selected from the group consisting of a single bond, phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho, phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

**53.** The method of introducing a nucleic acid into a cell of claim **51**,  
wherein

Z is a member selected from the group consisting of a single bond, phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho, phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

**54.** The method of introducing a nucleic acid into a cell of claim **51**,  
wherein

A is a diacylglycerolyl moiety;

X is phosphoethanolamido;

Z is NR, wherein R is a hydrogen atom; and

Y is a member selected from the group consisting of about 1 to about 10 basic amino acids or derivatives thereof.

**55.** The method of introducing a nucleic acid into a cell of claim **54**,  
wherein

A is a diacylglycerolyl moiety having 2 fatty acyl chains, wherein each acyl chain is independently between 2 and 30 carbons in length and is either saturated or has varying degrees of saturation.

1                   **56.**     The method of introducing a nucleic acid into a cell of claim **54**,  
2     wherein

3                   Y is a member selected from the group consisting of lysine, arginine,  
4     asparagine, glutamine, derivatives thereof and combinations thereof.

1                   **57.**     The method of introducing a nucleic acid into a cell of claim **54**,  
2     wherein

3                   A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl  
4     chain is a saturated C-18 carbon chain; and

5                   Y is a cationic group having 4 lysine residues or derivatives thereof.

1                   **58.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said conjugated lipid that inhibits aggregation of particles is a PEG-lipid.

1                   **59.**     The method of introducing a nucleic acid into a cell of claim **58**,  
2     wherein said PEG-lipid is PEG-ceramide.

1                   **60.**     The method of introducing a nucleic acid into a cell of claim **59**,  
2     wherein the ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to  
3     about 20 carbon atoms.

1                   **61.**     The method of introducing a nucleic acid into a cell of claim **59**,  
2     wherein said PEG-lipid is PEG-phosphatidylethanolamine.

1                   **62.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.

1                   **63.**     The method of introducing a nucleic acid into a cell of claim **33**,  
2     wherein said nucleic acid is selected from the group consisting of a plasmid, an antisense  
3     oligonucleotide, and a ribozyme.

1                   **64.**     A method for inducing  $H_{II}$  phase structure in a lipid bilayer, said  
2     method comprising: contacting said lipid bilayer with an endosomal membrane destabilizer,  
3     thereby inducing  $H_{II}$  phase structure in a lipid bilayer.

1                   **65.**     The method for inducing  $H_{II}$  phase structure of claim **64**, wherein said  
2     lipid bilayer comprises DOPC:DOPE:DOPS:Chol.



1                   **66.**     The method for inducing H<sub>II</sub> phase structure of claim **64**, wherein said  
2     endosomal membrane destabilizer is Ca<sup>++</sup> ion.

1                   **67.**     The method for inducing H<sub>II</sub> phase structure of claim **66**, wherein Ca<sup>++</sup>  
2     ion acts in concert with low levels of the cationic lipid to trigger H<sub>II</sub> phase formation.

1                   **68.**     Use of nucleic acid-lipid particle composition for introducing a nucleic  
2     acid into a cell, said particle comprising: a cationic lipid, a conjugated lipid that inhibits  
3     aggregation of particles, a nucleic acid and an endosomal membrane destabilizer.